USDA ARS National Animal Germplasm Program

Chicken Primordial Germ Cell (PGC) Collection and Cryopreservation Protocol

Collection:

Collect and pool gonads (n = 10 embryos) from 5.5 day old chicken embryos and place in a 1.5 mL siliconized tubes containing 500 µL of 10 % P-FBS DMEM.

Centrifuge the gonads at 600 x g for 5 minutes.

Dilute the gonad suspension with 200 µL of a 0.25 % trypsin/EDTA (Gibco, Grand Island, NY) solution and incubate for 20 minutes at 37 °C.

Tease the gonad suspension until only the homogenate remains and then remove it from the suspension.

Deactivate the trypsin with the addition of 200 µL of 20 % P-FBS DMEM.

Pass the cell suspension through a 30 micron filter that was initially saturated with 50 µL of 20 % P-FBS DMEM and collect the suspension in a 1.5mL siliconized tube.

Rinse the tube that held the trypsinized suspension twice with 200 µL of 20 % P-FBS DMEM and pass the rinsing solution through the filter as well.

Wash the tube again with 200 µL of 0% FBS DMEM twice and again pass the rinsing solutions through the filter.

Centrifuge the cell suspension for 5 minutes at 600 x g and remove the supernatant.

A 500 µL of 20 % ES-FBS 199 to the cell suspension.

The final PGC concentration from 10 embryos should be approximately 2 X 10^6 cells/mL.

Cool the cell suspension to 5 °C over one hour.

The remaining steps are performed at 5 °C.

Centrifuge the cell suspension for 5 minutes at 600 x g and remove the supernatant.

Dilute the cell suspension with 150 µL of 5 °C cryopreservation media, loaded into 0.5 mL straws and seal.

Cryopreservation and thawing:

Freeze the samples using a programmable freezer (e.g. Mini Digitcool UJ40, Cryo Bio System, Paris, France) and the following freeze curve: 5 °C to -85 °C at 1 °C/min and plunge into liquid nitrogen for storage.

Thaw straws for 30 seconds in a 37 °C water bath.

Recipes:

0 % FBS DMEM
1 % Penicillin/Streptomycin
99 % Dulbecco's Modified Eagle's Medium

10 % P-FBS DMEM
10 % Premium Fetal Bovine Serum
1 % Penicillin/Streptomycin
89 % Dulbecco's Modified Eagle's Medium

20 % P-FBS DMEM
20 % Premium Fetal Bovine Serum
1 % Penicillin/Streptomycin
79 % Dulbecco's Modified Eagle's Medium

20 % ES-FBS 199
20 % Embryonic Stem Cell-Qualified Fetal Bovine Serum
1 % Penicillin/Streptomycin
79 % Media 199

Cryopreservation medium:
20 % Embryonic Stem Cell-Qualified Fetal Bovine Serum
1 % Penicillin/Streptomycin
10 % Ethylene Glycol
69 % Medium 199

References:

Moore, D.T., P.H. Purdy, and H.D. Blackburn. 2006. A Method for Preserving Chicken Primordial Germ Cells. Poult. Sci. 85: 1784-1790.

Mozdziak, P.E., J. Angerman-Stewart, B. Rushton, S.L. Pardue, and J.N. Petitte. 2005. Isolation of chicken primordial germ cells using fluorescence-activated cell sorting. Poult. Sci. 84: 594-600.

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